REMARKS

Claims 1, 138, and 143 have been amended. Claims 134-137 and 141-142 have been cancelled. Claims 1, 60, 61, 130, 133, 138-140, and 143-145 are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance.

I. The Rejection of Claims 1, 60, 61, and 133-151 under 35 U.S.C. § 112, Second Paragraph

Claims 1, 60, 61, and 133-151 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for recitation of the phrase "microbial trypsin" because it is unclear whether said phrase refers to a wild-type microbial trypsin or some other genus of microbial trypsin molecules. This rejection is respectfully traversed for the reasons of record and further for the reasons below.

Applicants have previously indicated that the specification on page 4, line 34, to page 5, line 7, provides that the microbial trypsin may be a naturally occurring (wild-type) polypeptide, or it may be a variant thereof. Applicants use the term "microbial trypsin" throughout the specification. One of ordinary skill in the art would clearly understand that a microbial trypsin is obtained from a microbial source such as *Fusarium oxysporum*. This is in contrast to a mammalian trypsin that is obtained from a mammalian source. Such a microbial trypsin obtained from a microbial source would be understood in the art to be naturally occurring and consequently designated a wild-type microbial trypsin. However, Applicants also indicate that the microbial trypsin may even be a variant of a naturally occuring microbial trypsin, prepared by any suitable means. Consequently, Applicants submit that the term "microbial trypsin" is clear. However, if the Office has a suggestion on how to better phrase the term, Applicants would be appreciative to receive the suggestion.

For the foregoing reason, Applicants submit that the claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

II. The Rejection of Claims 1, 60, 61, and 133-151 under 35 U.S.C. § 112, First Paragraph

Claims 1, 60, 61, and 133-151 stand rejected under 35 U.S.C. § 112, first paragraph, as

failing to comply with the enablement requirement for the reasons previously stated of record. The Office Action previously stated:

> [T]he specification does not enable the skilled artisan to make and use the full scope of all variants of any microbial trypsin, wherein the variant has chymotrypsin-like activity and has, relative to SEQ ID NO: 2, a substitution at one or more of residues corresponding to 144, 193, 198, 201, 218, 223, or 227-231, a deletion at one or more of residues corresponding to 192, 197, or 226, an insertion between residues 224 and 225, and has either 70% homology to residues 25-248 of SEQ ID NO: 2 or hybridizes at low stringency to residues 202-801 of SEQ ID NO: 1.

This rejection is respectfully traversed for the reasons of record and further for the reasons described below, which were presented in the Amendment of August 14, 2006 but were not addressed by the Office in the Advisory Action of September 26, 2006.

The Office asserts that it would require undue experimentation for the skilled artisan to isolate and test all variants for chymotrypsin activity because the number of polypeptides encompassed by the phrase "a microbial trypsin" is extremely large, including both any wild-type microbial trypsin and any naturally-occurring or recombinant variant thereof having trypsin activity. Applicants disagree with this assertion.

The issue is whether in an unpredictable art, Section 112 requires disclosure of a test with every species covered by a claim. To require such a complete disclosure would apparently necessitate a patent application or applications with "hundreds" of variants along with information as to whether each microbial trypsin variant has chymotrypsin-like activity. More importantly, such a requirement would force an inventor seeking adequate patent protection to carry out a prohibitive number of actual experiments. This would tend to discourage inventors from filing patent applications in an unpredictable area since the patent claims would have to be limited to those embodiments which are expressly disclosed. A potential infringer could readily avoid "literal" infringement of such claims by merely using a different microbial trypsin and constructing another variant thereof having chymotrypsin-like activity using Applicants' specification.

If one skilled in this art wished to construct a microbial trypsin variant having chymotrypsin-like activity using a different microbial trypsin from the one disclosed in Applicants' specification, he would merely read Applicants' specification for directions how to make such a variant. Applicants' claimed invention is not complicated, and no special equipment or unusual procedures must be provided when practicing the invention. The methods for using another microbial trypsin to construct such a variant having chymotrypsin activity is well described in Applicants' specification. While some experimentation is necessary to isolate and test other

variants for chymotrypsin activity, such experimentation is a simple process and well known in the art. The mere fact that the experimentation may be time-consuming, as alleged by the Office, does not mandate a conclusion that such experimentation is undue in the art. In fact, the effort required to isolate and test other variants for chymotrypsin activity is ordinary in the field of constructing and testing variants and certainly does not require ingenuity beyond that expected of one of ordinary skill in the art. The test is not quantitative, since a considerable amount of experimentation is permissible, since it is merely routine. Moreover, the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice the claimed invention. Thus, there is no basis for concluding that it would be undue experimentation for the skilled artisan to isolate and test other variants for chymotrypsin activity.

The Office also asserts that all microbial trypsins could not be successfully aligned with SEQ ID NO: 2 in a manner to identify residues corresponding to 144, 192, 193, 197, 198, 201, 218, 223-225, and 226-231. Applicants disagree with this assertion. The Office provides no evidence to support such a contention and, consequently, has not met its burden.

Applicants submit that all microbial trypsins could be successfully aligned with SEQ ID NO: 2 in a manner to identify residues corresponding to 144, 192, 193, 197, 198, 201, 218, 223-225, and 226-231. Applicants describe the catalytic region and the amino acid positions of a microbial trypsin protein required to be mutated to produce a microbial trypsin variant having chymotrypsin-like activity using amino acids 25 to 248 of SEQ ID NO: 2 as a reference protein. These mutations include (1) substitutions at positions corresponding to positions 144, 193, 198, 201, 218, 223, 227, 228, 229, 230, and 231 of amino acids 25 to 248 of SEQ ID NO: 2, (2) deletions at positions corresponding to positions 192, 197, and 226 of amino acids 25 to 248 of SEQ ID NO: 2; and (3) an insertion between positions corresponding to positions 224 and 225 of amino acids 25 to 248 of SEQ ID NO: 2.

Identification of corresponding amino acids in another microbial trypsin is accomplished by aligning the amino acid sequence of the microbial trypsin with the amino acid sequences of one or more chymotrypsins and/or by comparing the secondary or 3D structures of the microbial trypsin and one or more chymotrypsins including the microbial trypsin variant having chymotrypsin activity of the present invention (see page 13, lines 11-26 of the specification). Applicants provide details on page 6, line 2, to page 7, line 9, of the specification of how to indicate the position of an amino acid residue in a microbial trypsin in regions of structural homology using the numbering system originating from the amino acid sequence of the microbial trypsin disclosed in SEQ ID NO: 2, aligned with the amino acid sequence of another microbial

trypsin. Applicants also disclose methods and examples for the construction of variants with chymotrypsin-like activity from a microbial trypsin by identifying the positions of the amino acids in the microbial trypsin that correspond to the amino acids of a chymotrypsin responsible for catalytic activity and substituting, deleting, and/or inserting amino acids in the microbial trypsin to correspond to the same and/or similar amino acids of the chymotrypsin by site-directed mutagenesis or any other suitable method known in the art. The amino acids of a chymotrypsin responsible for catalytic activity are well known in the art and include not only the amino acids involved in enzyme catalysis, but also the amino acids of the binding site and surface loops of the binding pocket.

Again, the test is not quantitative, since a considerable amount of experimentation is permissible, since it is merely routine. Moreover, the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice the claimed invention.

The Office also asserts that the full scope of the invention requires further undue experimentation to identify and test, for chymotrypsin activity, all polypeptides having the designated substitutions, deletions, and insertion and either at least 70% homology to residues 25-248 of SEQ ID NO: 2 or hybridizes at low stringency to residues 202-801 of SEQ ID NO: 1. The Office cites Guo *et al.*, 2004, *PNAS USA* 101: 9205-9210, to support its assertion. Applicants disagree with this assertion for the reasons described above. Moreover, the citation of Guo *et al.* is irrelevant to the instant invention because the citation involves random mutations of a protein. This is not the situation in the instant invention. Applicants describe the catalytic region and the amino acid positions of a microbial trypsin protein required to be mutated to produce a microbial trypsin variant having chymotrypsin-like activity using amino acids 25 to 248 of SEQ ID NO: 2 as a reference protein. However, to further prosecution of the instant application, the claims have been amended to recite in part: "the microbial trypsin is (a) a polypeptide comprising an amino acid sequence which has at least 90% identity to amino acids 25 to 248 of SEQ ID NO: 2; or (b) a polypeptide encoded by a nucleotide sequence which hybridizes under at least medium-high stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or its complementary strand ..."

The Office also asserts that while enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed and sufficient guidance has not been provided in the instant specification. Applicants disagree with this assertion when applied to the instant application.

Applicants submit that the specification provides a rational and predictable scheme for modifying the designated amino acid residues with an expectation of obtaining microbial trypsin variants having chymotrypsin-like activity. The specification describes methods for obtaining microbial trypsin-like or trypsinogen-like proteins on page 9, line 21, to page 13, line 14; methods for identifying the positions of the amino acids in the microbial trypsin that correspond to the amino acids of a chymotrypsin responsible for catalytic activity on page 6, line 1, to page 7, line 9; methods for constructing microbial trypsin variants with chymotrypsin-like activity using various techniques known in the art such as site-directed mutagenesis on page 13, line 16, to page 18, line 12, and Example 1; methods for expressing the microbial trypsin variants having chymotrypsin-like activity on page 29, line 15, to page 40, line 11, and Examples 2 and 3; methods for assaying for trypsin and chymotrypsin activity on page 4, line 28, to page 5, line 1, and page 5, line 13-20; and methods for purifying and characterizing the microbial trypsin variants having chymotrypsin-like activity in Examples 4-7. The specification is very clear about what amino acid positions to mutate as described above. In fact, Applicants in Example 1 describe how to mutate these positions and show in later Examples that a microbial trypsin variant has chymotrypsin-like activity. The Examiner's conclusions fail to give appropriate consideration to the high level of skill in the art. As of the time of the invention, it was routine for persons of ordinary skill in the art to prepare and screen for variants of SEQ ID NO: 2 which encode a protein that possess at least 70% identity to SEQ ID NO: 2 and has chymotrypsin-like activity.

Moreover, the information in the public domain also provides detailed guidance to assist an artisan when preparing microbial trypsin variants having chymotrypsin-like activity including detailed guidance of both conserved sequences, sequences which are important for function, and sequences which can be altered without disrupting chymotrypsin activity, as well as detailed information of the ways in which the protein's structure relates to its function. In particular, Hedstrom *et al.*, 1992, *Science* 255: 1240-1253, disclose the protein engineering of a mammalian trypsin gene to code for a polypeptide with a functional chymotrypsin substrate profile by site-directed mutagenesis of the S1 binding site and surface loops of the binding pocket of trypsin with analogous residues of chymotrypsin.

This teaching, coupled with Applicants' specification and examples and the ability to test for functional mutants with the assays provided in the specification, provide sufficient guidance to enable an artisan to make the mutations disclosed for SEQ ID NO: 2 in other microbial trypsins. Such work is certainly not undue, as the production of variants using this technology was routine in the art as of the filing of the application.

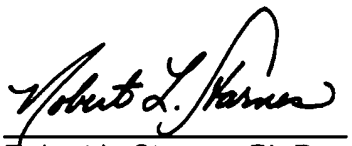
Accordingly, the specification enables the claimed invention because the specification contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,



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Robert L. Starnes, Ph.D.
Reg. No. 41,324
Novozymes, Inc.
1445 Drew Avenue
Davis, CA 95616
(530) 757-8100